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L2: Entry 8 of 15

File: USPT

Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358726 B1

TITLE: Thermostable protease

Brief Summary Text (19):

wherein SIG represents an amino acid sequence of a signal peptide derived from a subtilisin, PRO represents an amino acid sequence of a protein to be expressed. Preferably, SIG is the amino acid sequence represented by the SEQ ID NO:3 of the Sequence Listing. Preferably, PRO is an amino acid sequence of a hyperthermostable protease derived from a hyperthermophile, more preferably, an amino acid sequence of a protease derived from Pyrococcus furiosus.

Detailed Description Text (2):

The hyperthermostable protease according to the present invention includes proteases from various hyperthermophiles. For example, WO 95/34645 describes proteases from Pyrococcus furiosus and Thermococcus celer.

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All: 1 Review: 0 ☒

☐ 1: Curr Biol. 1996 Jun 1;6(6):739-49.

Cell Press Links

A hyperthermostable protease of the subtilisin family bound to the surface layer of the archaeon *Staphylothermus marinus*.

Mayr J, Lupas A, Kellermann J, Eckerskorn C, Baumeister W, Peters J.

Max-Planck-Institut für Biochemie, Martinsried, Germany.

BACKGROUND: *Staphylothermus marinus*, an archaeon isolated from a geothermally heated marine environment, is a peptide-fermenting, sulphur-dependent organism with an optimum growth temperature of 92 degrees C. It forms grapes of cells, which adhere to each other and to sulphur granules via their surface layer. This glycoprotein layer forms a canopy which is held at a distance of about 70 nm from the cell membrane by membrane-anchored stalks, thereby enclosing a 'quasi-periplasmic space'. Two copies of a globular protease, which probably serves an exodigestive function related to the organism's energy metabolism, are attached near the middle of each stalk. **RESULTS:** We have purified and characterized this protease with regard to its enzymatic properties and thermostability, and have sequenced its gene using an approach based entirely on the polymerase chain reaction. The precursor form is 1345 amino acids long; between residues 64-741, it contains a domain with clear homology to subtilisins, which is interrupted by two large insertions. The enzyme has a broad substrate specificity and a pH optimum of 9.0. It is fully stable from pH 3.2 to 12.7 and is resistant to heat-inactivation to 95 degrees C in the free form and to 125 degrees C in the stalk-bound form.

CONCLUSIONS: This protease is one of the most stable proteases known. Its high resistance towards denaturing agents makes it an interesting target for practical applications. Despite its large size, it is clearly a member of the subtilisin family and represents the only known enzyme that is a stoichiometric S-layer component.

PMID: 8793300 [PubMed - indexed for MEDLINE]

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Hyperthermostable surface layer protein tetrabrachion from the archaeobacterium *Staphylothermus marinus*: evidence for the presence of a right-handed coiled coil derived from the primary structure [J Biol Chem. 1996]

Active subtilisin-like protease from a hyperthermophilic archaeon in a form with a unique precursor [Appl Microbiol. 2001]

A novel member of the subtilisin-like protease family from *Streptomyces albogriseolus*. [J Bacteriol. 1997]

Isolation and characterization of the hyperthermostable serine protease, pyrolysin, and its gene from the hyperthermophilic archaeon *Pyrococcus furiosus* [J Biol Chem. 1996]

Homology modelling of two subtilisin-like proteases from the hyperthermophilic archaea *Pyrococcus furiosus* and *Thermococcus stettensis* [Protein Eng. 1997]

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(L1 SAME PYROCOCCUS FURIOSUS).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	15

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L2 L1 same pyrococcus furiosus
L1 hyperthermostable protease

15 L2
 21 L1

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Search Results - Record(s) 1 through 15 of 15 returned.

☐ 1. Document ID: US 20050084934 A1

L2: Entry 1 of 15

File: PGPB

Apr 21, 2005

PGPUB-DOCUMENT-NUMBER: 20050084934

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050084934 A1

TITLE: System for expressing hyperthermostable protein

PUBLICATION-DATE: April 21, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Takakura, Hikaru	Otsu-shi		JP
Morishita, Mio	Otsu-shi		JP
Shimojo, Tomoko	Kyoto-shi		JP
Asada, Kiyozo	Koka-gun		JP
Kato, Ikunoshin	Uji-shi		JP

US-CL-CURRENT: [435/69.1](#); [435/220](#), [435/252.3](#), [435/320.1](#), [510/320](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw D
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☐ 2. Document ID: US 20050014221 A1

L2: Entry 2 of 15

File: PGPB

Jan 20, 2005

PGPUB-DOCUMENT-NUMBER: 20050014221

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050014221 A1

TITLE: Hyperthermostable protease gene

PUBLICATION-DATE: January 20, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Takakura, Hikaru	Otsu-shi		JP
Morishita, Mio	Otsu-shi		JP
Yamamoto, Katsuhiko	Otsu-shi		JP
Mitta, Masanori	Kyotanabe-shi		JP

Asada, Kiyozo	Koka-gun	JP
Tsunasawa, Susumu	Otsu-shi	JP
Kato, Ikunoshin	Uji-shi	JP

US-CL-CURRENT: [435/69.1](#); [435/226](#), [435/252.3](#), [435/320.1](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw De
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☐ 3. Document ID: US 20020132335 A1

L2: Entry 3 of 15

File: PGPB

Sep 19, 2002

PGPUB-DOCUMENT-NUMBER: 20020132335
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020132335 A1

TITLE: System for expressing hyperthermostable protein

PUBLICATION-DATE: September 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Takakura, Hikaru	Otsu-shi		JP
Morishita, Mio	Otsu-shi		JP
Shimojo, Tomoko	Kyoto-shi		JP
Asada, Kiyozo	Koka-gun		JP
Kato, Ikunoshin	Uji-shi		JP

US-CL-CURRENT: [435/226](#); [435/219](#), [435/252.31](#), [435/320.1](#), [435/69.1](#), [536/23.2](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMC	Draw De
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☐ 4. Document ID: US 20020086402 A1

L2: Entry 4 of 15

File: PGPB

Jul 4, 2002

PGPUB-DOCUMENT-NUMBER: 20020086402
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020086402 A1

TITLE: Hyperthermostable protease gene

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Takakura, Hikaru	Otsu-shi		JP
Morishita, Mio	Otsu-shi		JP
Yamamoto, Katsuhiko	Otsu-shi		JP

Mitta, Masanori	Kyotanabe-shi	JP
Asada, Kiyozo	Koka-gun	JP
Tsunasawa, Susumu	Otsu-shi	JP
Kato, Ikunoshin	Uji-shi	JP

US-CL-CURRENT: 435/226; 435/325, 435/69.1, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC	Draw. De
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☐ 5. Document ID: US 7144719 B2

L2: Entry 5 of 15

File: USPT

Dec 5, 2006

US-PAT-NO: 7144719

DOCUMENT-IDENTIFIER: US 7144719 B2

TITLE: Method for producing protease

PRIOR-PUBLICATION:

DOC-ID

DATE

US 20050084934 A1

April 21, 2005

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC	Draw. De
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☐ 6. Document ID: US 6849441 B2

L2: Entry 6 of 15

File: USPT

Feb 1, 2005

US-PAT-NO: 6849441

DOCUMENT-IDENTIFIER: US 6849441 B2

TITLE: Hyperthermostable protease gene

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC	Draw. De
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☐ 7. Document ID: US 6783970 B2

L2: Entry 7 of 15

File: USPT

Aug 31, 2004

US-PAT-NO: 6783970

DOCUMENT-IDENTIFIER: US 6783970 B2

TITLE: System for expressing hyperthermostable protein

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWC	Draw. De
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☐ 8. Document ID: US 6358726 B1

L2: Entry 8 of 15

File: USPT

Mar 19, 2002

US-PAT-NO: 6358726

DOCUMENT-IDENTIFIER: US 6358726 B1

TITLE: Thermostable protease

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 9. Document ID: US 6261822 B1

L2: Entry 9 of 15

File: USPT

Jul 17, 2001

US-PAT-NO: 6261822

DOCUMENT-IDENTIFIER: US 6261822 B1

TITLE: Ultrathermostable protease genes

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 10. Document ID: US 5756339 A

L2: Entry 10 of 15

File: USPT

May 26, 1998

US-PAT-NO: 5756339

DOCUMENT-IDENTIFIER: US 5756339 A

TITLE: Hyperthermostable protease gene

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 11. Document ID: JP 2004329217 A

L2: Entry 11 of 15

File: JPAB

Nov 25, 2004

PUB-NO: JP02004329217A

DOCUMENT-IDENTIFIER: JP 2004329217 A

TITLE: HYPERTHERMOSTABLE PROTEASE GENE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw De
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☐ 12. Document ID: EP 776971 A1

L2: Entry 12 of 15

File: EPAB

Jun 4, 1997

PUB-NO: EP000776971A1

DOCUMENT-IDENTIFIER: EP 776971 A1

TITLE: HYPERTHERMOSTABLE PROTEASE GENE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw. De
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☐ 13. Document ID: WO 9534645 A1

L2: Entry 13 of 15

File: EPAB

Dec 21, 1995

PUB-NO: WO009534645A1

DOCUMENT-IDENTIFIER: WO 9534645 A1

TITLE: HYPERTHERMOSTABLE PROTEASE GENE

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw. De
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☐ 14. Document ID: KR 530598 B, WO 9856926 A1, AU 9875500 A, EP 994191 A1, CN 1260002 A, JP 11502065 X, KR 2001013540 A, US 6358726 B1, US 20020132335 A1, TW 530088 A, US 6783970 B2, JP 3601835 B2, US 20050084934 A1, CN 1657614 A, EP 994191 B1, CN 1190494 C, DE 69833652 E, DE 69833652 T2, US 7144719 B2

L2: Entry 14 of 15

File: DWPI

Nov 23, 2005

DERWENT-ACC-NO: 1999-080907

DERWENT-WEEK: 200682

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TITLE: Recombinant hyperthermostable protease from Pyrococcus furiosus - and gene encoding it, for large scale production of the protease for industrial use.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw. De
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☐ 15. Document ID: JP 3708946 B2, WO 9534645 A1, JP 08501922 X, EP 776971 A1, US 5756339 A, EP 776971 A4, EP 776971 B1, DE 69524422 E, CN 1154717 A, JP 2004329217 A, CN 1086737 C

L2: Entry 15 of 15

File: DWPI

Oct 19, 2005

DERWENT-ACC-NO: 1996-049674

DERWENT-WEEK: 200569

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TITLE: Pyrococcus furiosus hyper:thermostable protease gene - useful for recombinant prodn. of hyper:thermostable protease

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequence	Attachments	Claims	KWIC	Draw. De
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Term	Documents
PYROCOCCUS	1721

PYROCOCCU	3
FURIOSUS	1155
FURIOSU	0
(1 SAME (PYROCOCCUS ADJ FURIOSUS)).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	15
(L1 SAME PYROCOCCUS FURIOSUS).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	15

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SEA (HYPERTHERMOSTABLE PROTEASE)

1 FILE AQUASCI
7 FILE BIOSIS
1 FILE BIOTECHABS
1 FILE BIOTECHDS
1 FILE BIOTECHNO
5 FILE CAPLUS
5 FILE CEABA-VTB
1 FILE CIN
49 FILE DGENE
1 FILE EMBASE
1 FILE FROSTI
67 FILE GENBANK
10 FILE IFIPAT
1 FILE JICST-EPLUS
1 FILE LIFESCI
1 FILE MEDLINE
1 FILE PROMT
2 FILE SCISEARCH
9 FILE USPATFULL
4 FILE USPAT2
2 FILE WPIDS
1 FILE WPIFV
2 FILE WPINDEX
L1 QUE (HYPERTHERMOSTABLE PROTEASE)

FILE 'DGENE, IFIPAT, USPATFULL, BIOSIS, CAPLUS, CEABA-VTB, USPAT2, SCISEARCH, WPIDS, AQUASCI, BIOTECHDS, BIOTECHNO' ENTERED AT 15:11:51 ON 09 JAN 2007

L2 77 S L1 AND PYROCOCCLUS
L3 65 DUP REM L2 (12 DUPLICATES REMOVED)
L4 11 S L3 AND (ISOLAT? OR PURIF?)

=> d 14 ibib ab 1-11

L4 ANSWER 1 OF 11 IFIPAT COPYRIGHT 2007 IFI on STN
AN 10775515 IFIPAT;IFIUDB;IFICDB
TITLE: **HYPERTHERMOSTABLE PROTEASE GENE**
INVENTOR(S): Asada; Kiyozo, Koka-gun, JP
Kato; Ikunoshin, Uji-shi, JP
Mitta; Masanori, Kyotanabe-shi, JP
Morishita; Mio, Otsu-shi, JP
Takakura; Hikaru, Otsu-shi, JP
Tsunasawa; Susumu, Otsu-shi, JP
Yamamoto; Katsuhiko, Otsu-shi, JP
PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Kyoto-shi, JP
AGENT: BROWDY AND NEIMARK, P.L.L.C.; 624 NINTH STREET, NW,
SUITE 300, WASHINGTON, DC, 20001-5303, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2005014221	A1	20050120
APPLICATION INFORMATION:	US 2004-800684		20040316

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
DIVISION OF:	US 1998-894818	19980520	6261822
DIVISION OF:	US 2001-841553	20010424	PENDING

	NUMBER	DATE
PRIORITY APPLN. INFO.:	JP 1995-323285	19951212
FAMILY INFORMATION:	US 2005014221	20050120
	US 6261822	
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	
FILE SEGMENT:	CHEMICAL	
	APPLICATION	

PARENT CASE DATA:

This is a divisional of application Ser. No. 09/841,553, filed Apr. 24, 2001, which is a divisional of application Ser. No. 08/ 894,818, now issued as U.S. Pat. No. 6,261,822, which is a 371 national stage application of PCT/JP96/03253, filed Nov. 7, 1996, the entire contents of both applications being incorporated herein by reference.

NUMBER OF CLAIMS: 6 32 Figure(s).
DESCRIPTION OF FIGURES:

FIG. 1 is a figure showing a restriction map of a DNA fragment derived from ***Pyrococcus*** furiosus contained in the plasmid pTPR12 and the plasmid pUBP13.

FIG. 2 is a figure showing a design of the oligonucleotide PRO1F (SEQ ID NO:9) based on nucleotides 628 to 669 of SEQ ID NO:7 which encode residues 169 to 182 of SEQ ID NO:8.

FIG. 3 is a figure showing a design of the oligonucleotide PRO2F (SEQ ID NO:10) based on nucleotides 1210 to 1251 of SEQ ID NO:7 which encode residues 363 to 376 of SEQ ID NO:8 and PRO-2R (SEQ ID NO:11).

FIG. 4 is a figure showing a design of the oligonucleotide PRO4R (SEQ ID NO:12) based on nucleotides 1882 to 1923 of SEQ ID NO:7 which encode residues 587 to 600 of SEQ ID NO:8.

FIG. 5 is a restriction map of the plasmid p2F-4R.

FIG. 6 is a restriction map of the plasmid pTC3.

FIG. 7 is a restriction map of the plasmid pTCS6.

FIG. 8 is a restriction map of the plasmid pTC4.

FIG. 9 is a figure showing the procedures for constructing the plasmid pSTC3.
 FIG. 10 is a restriction map of the plasmid pSTC3.
 FIG. 11 is a figure comparing the amino acid sequences of the various proteases of PFUL (SEQ ID NO:8), TCES (SEQ ID NO:1) and Subtilisin (SEQ ID NO:45).
 FIG. 12 is a continuation of FIG. 11.
 FIG. 13 is a figure showing a restriction map around the protease PFUS gene on the *Pyrococcus furiosus* chromosomal DNA.
 FIG. 14 is a restriction map of the plasmid pSPT1.
 FIG. 15 is a restriction map of the plasmid pSNP1.
 FIG. 16 is a restriction map of the plasmid pPS1.
 FIG. 17 is a restriction map of the plasmid pNAPS1.
 FIG. 18 is a figure showing the optimum temperature for the enzyme preparation TC-3.
 FIG. 19 is a figure showing the optimum temperature for the enzyme preparation NAPS-1.
 FIG. 20 is a figure showing the optimum pH for the enzyme preparation TC-3.
 FIG. 21 is a figure showing the optimum pH for the enzyme preparation NP-1.
 FIG. 22 is a figure showing the optimum pH for the enzyme preparation NAPS-1.
 FIG. 23 is a figure showing the thermostability of the enzyme preparation TC-3.
 FIG. 24 is a figure showing the thermostability of the enzyme preparation NP-1.
 FIG. 25 is a figure showing the thermostability of the activated enzyme preparation NP-1.
 FIG. 26 is a figure showing the thermostability of the enzyme preparation NAPS-1.
 FIG. 27 is a figure showing the pH-stability of the enzyme preparation NP-1.
 FIG. 28 is a figure showing the stability of the enzyme preparation NP-1 in the presence of SDS.
 FIG. 29 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of SDS.
 FIG. 30 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of acetonitrile.
 FIG. 31 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of urea.
 FIG. 32 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of guanidine hydrochloride.

AB There are provided **hyperthermostable proteases** having an amino acid sequences represented by SEQ ID Nos. 1, 3 and 5 of the Sequence Listing or functional equivalents thereof and **hyperthermostable protease** genes encoding those **hyperthermostable protease**. There is also disclosed a process for preparation of a **hyperthermostable protease** by culturing a transformant containing the gene.

L4 ANSWER 2 OF 11 IFIPAT COPYRIGHT 2007 IFI on STN
 AN 10142763 IFIPAT;IFIUDB;IFICDB
 TITLE: **HYPERTHERMOSTABLE PROTEASE GENE;**
HEAT RESISTANT ENZYMATIC POLYPEPIDE; FOR USE AS TOOL
IN GENETIC ENGINEERING
 INVENTOR(S): Asada; Kiyozo, Koka-gun, JP
 Kato; Ikunoshin, Uji-shi, JP
 Mitta; Masanori, Kyotanabe-shi, JP
 Morishita; Mio, Otsu-shi, JP
 Takakura; Hikaru, Otsu-shi, JP
 Tsunasawa; Susumu, Otsu-shi, JP
 Yamamoto; Katsuhiko, Otsu-shi, JP
 PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Kyoto-shi, JP
 AGENT: BROWDY AND NEIMARK, P.L.L.C. PATENT AND TRADEMARK
 CAUSES, SUITE 300, 624 NINTH STREET, N.W.,
 WASHINGTON, DC, 20001-5303, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002086402	A1	20020704
APPLICATION INFORMATION:	US 2001-841553		20010424

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
Section 371 PCT Filing OF:	WO 1996-JP3253	19961107	UNKNOWN
DIVISION OF:	US 1998-894818	19980520	6261822

	NUMBER	DATE
PRIORITY APPLN. INFO.:	JP 1995-323285	19951212
FAMILY INFORMATION:	US 2002086402	20020704
	US 6261822	
	US 6849441	20050201
DOCUMENT TYPE:	Utility	
	Patent Application - First Publication	
FILE SEGMENT:	CHEMICAL APPLICATION	

NUMBER OF CLAIMS: 10 32 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 is a figure showing a restriction map of a DNA fragment derived from ***Pyrococcus*** furiosus contained in the plasmid pTPR12 and the plasmid PUBP13.

FIG. 2 is a figure showing a design of the oligonucleotide PRO1F.

FIG. 3 is a figure showing a design of the oligonucleotide PRO2F and PRO-2R.

FIG. 4 is a figure showing a design of the oligonucleotide PRO4R.

FIG. 5 is a restriction map of the plasmid p2F-4R.

FIG. 6 is a restriction map of the plasmid pTC3.

FIG. 7 is a restriction map of the plasmid pTCS6.

FIG. 8 is a restriction map of the plasmid pTC4.

FIG. 9 is a figure showing the procedures for constructing the plasmid pSTC3.

FIG. 10 is a restriction map of the plasmid pSTC3.

FIG. 11 is a figure comparing the amino acid sequences of the various proteases.

FIG. 12 is a continuation of FIG. 11.

FIG. 13 is a figure showing a restriction map around the protease PFUS gene on the *Pyrococcus furiosus* chromosomal DNA.

FIG. 14 is a restriction map of the plasmid pSPT1.

FIG. 15 is a restriction map of the plasmid pSNP1.

FIG. 16 is a restriction map of the plasmid pPS1.

FIG. 17 is a restriction map of the plasmid pNAPS1.

FIG. 18 is a figure showing the optimum temperature for the enzyme preparation TC-3.

FIG. 19 is a figure showing the optimum temperature for the enzyme preparation NAPS-1.

FIG. 20 is a figure showing the optimum pH for the enzyme preparation TC-3.

FIG. 21 is a figure showing the optimum pH for the enzyme preparation NP-1.

FIG. 22 is a figure showing the optimum pH for the enzyme preparation NAPS-1.

FIG. 23 is a figure showing the thermostability of the enzyme preparation TC-3.

FIG. 24 is a figure showing the thermostability of the enzyme preparation NP-1.

FIG. 25 is a figure showing the thermostability of the activated enzyme preparation NP-1.

FIG. 26 is a figure showing the thermostability of the enzyme preparation NAPS-1.

FIG. 27 is a figure showing the pH-stability of the enzyme preparation NP-1.

FIG. 28 is a figure showing the stability of the enzyme preparation NP-1 in the presence of SDS.

FIG. 29 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of SDS.

FIG. 30 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of acetonitrile.

FIG. 31 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of urea.

FIG. 32 is a figure showing the stability of the enzyme preparation NAPS-1 in

the presence of guanidine hydrochloride.

AB There are provided **hyperthermostable proteases** having an amino acid sequences represented by SEQ ID NOS: 1, 3 and 5 of the Sequence Listing or functional equivalents thereof and **hyperthermostable protease** genes encoding those **hyperthermostable protease**. There is also disclosed a process for preparation of a **hyperthermostable protease** by culturing a transformant containing the gene.

L4 ANSWER 3 OF 11 IFIPAT COPYRIGHT 2007 IFI on STN

AN 04192605 IFIPAT;IFIUDB;IFICDB

TITLE: **HYPERTHERMOSTABLE PROTEASE GENE;**
HEAT RESISTANT ENZYMATIC POLYPEPIDE; FOR USE AS TOOL
IN GENETIC ENGINEERING

INVENTOR(S): Asada; Kiyozo, Shiga, JP
Kato; Ikunoshin, Uji, JP
Mitta; Masanori, Kyotanabe, JP
Morishita; Mio, Otsu, JP

Takakura; Hikaru, Otsu, JP
Tsunasawa; Susumu, Otsu, JP
Yamamoto; Katsuhiko, Otsu, JP
PATENT ASSIGNEE(S): Takara Shuzo Co., LTD, Kyoto, JP

PRIMARY EXAMINER: Rao, Manjunath

AGENT: Browdy and Neimark, PLLC

	NUMBER	PK	DATE
PATENT INFORMATION:	US 6849441	B2	20050201
	US 2002086402	A1	20020704
APPLICATION INFORMATION:	US 2001-841553		20010424
EXPIRATION DATE:	20 May 2018		

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
DIVISION OF:	US 1998-894818	19980520	6261822

	NUMBER	DATE
PRIORITY APPLN. INFO.:	JP 1995-323285	19951212
FAMILY INFORMATION:	US 6849441	20050201
	US 6261822	
	US 2002086402	20020704
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Granted Patent - Utility, with Pre-Grant Publication	
	CHEMICAL	
	GRANTED	

PARENT CASE DATA:

This application is a divisional of Ser. No. 08/894,818, filed May 20, 1998, now U.S. Pat. No. 6,261,822, issued on Jul. 17, 2001, which is a 371 of PCT/JP96/03253, filed Nov. 7, 1996.

NOTE: INDEXED FROM APPLICATION
Subject to any Disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by 267 days.

MICROFILM REEL NO: 014546 FRAME NO: 0568
014546 0587

NUMBER OF CLAIMS: 6

GRAPHICS INFORMATION: 24 Drawing Sheet(s), 32 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 is a figure showing a restriction map of a DNA fragment derived from ***Pyrococcus*** furiosus contained in the plasmid pTPR12 and the plasmid

pUBP13.

FIG. 2 is a figure showing a design of the oligonucleotide PRO1F (SEQ ID NO:9) based on nucleotides 628 to 669 of SEQ ID NO: 7) which encode residues 169 to 182 of SEQ ID NO:8.

FIG. 3 is a figure showing a design of the oligonucleotide PRO2F (SEQ ID NO:10) based on nucleotides 1210 to 1251 of SEQ ID NO:7 which encoded residues 363 to 376 of SEQ ID NO:8 and PRO2R (SEQ ID NO:11).

FIG. 4 is a figure showing a design of the oligonucleotide PRO4R (SEQ ID NO:12) based on nucleotides 1882 to 1923 of SEQ ID NO:7 which encode residues 587 to 600 of SEQ ID NO:8.

FIG. 5 is a restriction map of the plasmid p2F-4R.

FIG. 6 is a restriction map of the plasmid pTC3.

FIG. 7 is a restriction map of the plasmid pTCS6.

FIG. 8 is a restriction map of the plasmid pTC4.

FIG. 9 is a figure showing the procedures for constructing the plasmid pSTC3.

FIG. 10 is a restriction map of the plasmid pSTC3.

FIG. 11 is a figure comparing the amino acid sequences of the various proteases of PFUL (SEQ ID NO:8), TCES (SEQ ID NO:1) and subtilisin (SEQ ID NO:45).

FIG. 12 is a continuation of FIG. 11.

FIG. 13 is a figure showing a restriction map around the protease PFUS gene on the *Pyrococcus furiosus* chromosomal DNA.

FIG. 14 is a restriction map of the plasmid pSPT1.

FIG. 15 is a restriction map of the plasmid pSNP1.

FIG. 16 is a restriction map of the plasmid pPS1.

FIG. 17 is a restriction map of the plasmid pNAPS1.

FIG. 18 is a figure showing the optimum temperature for the enzyme preparation TC-3.

FIG. 19 is a figure showing the optimum temperature for the enzyme preparation NAPS-1.

FIG. 20 is a figure showing the optimum pH for the enzyme preparation TC-3.

FIG. 21 is a figure showing the optimum pH for the enzyme preparation NP-1.

FIG. 22 is a figure showing the optimum pH for the enzyme preparation NAPS-1.

FIG. 23 is a figure showing the thermostability of the enzyme preparation TC-3.

FIG. 24 is a figure showing the thermostability of the enzyme preparation NP-1.

FIG. 25 is a figure showing the thermostability of the activated enzyme preparation NP-1.

FIG. 26 is a figure showing the thermostability of the enzyme preparation NAPS-1.

FIG. 27 is a figure showing the pH-stability of the enzyme preparation NP-1.

FIG. 28 is a figure showing the stability of the enzyme preparation NP-1 in the presence of SDS.

FIG. 29 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of SDS.

FIG. 30 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of acetonitrile.

FIG. 31 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of urea.

FIG. 32 is a figure showing the stability of the enzyme preparation NAPS-1 in the presence of guanidine hydrochloride.

AB There are provided **hyperthermostable proteases** having an amino acid sequences represented by SEQ ID NOs: 1, 3 and 5 of the Sequence Listing or functional equivalents thereof and **hyperthermostable protease** genes encoding those **hyperthermostable protease**. There is also disclosed a process for preparation of a **hyperthermostable protease** by culturing a transformant containing the gene.

L4 ANSWER 4 OF 11 IFIPAT COPYRIGHT 2007 IFI on STN

AN 02984329 IFIPAT;IFIUDB;IFICDB

TITLE: **HYPERTHERMOSTABLE PROTEASE GENE;**
CULTURING A TRANSFORMANT TRANSFORMED WITH A PLASMID
INTO WHICH THE GENE HAS BEEN TRANSDUCE; FROM
PYROCOCCUS FURIOSUS

INVENTOR(S): Asada, Kiyozo, Koka-gun, JP

Kato, Ikunoshin, Uji, JP
 Mitta, Masanori, Tsuzuki-gun, JP
 Morishita, Mio, Otsu, JP
 Tsunasawa, Susumu, Kusatsu, JP
 Yamamoto, Katsuhiko, Otsu, JP
 PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Kyoto, JP
 PRIMARY EXAMINER: Wax, Robert A
 ASSISTANT EXAMINER: Stole, Einar
 AGENT: Browdy and Neimark

	NUMBER	PK	DATE
PATENT INFORMATION:	US 5756339	A	19980526
	(CITED IN 003 LATER PATENTS)		
	WO 9534645		19951221
APPLICATION INFORMATION:	US 1996-750532		19961213
	WO 1995-JP1095		19950605
			19961213 PCT 371 date
			19961213 PCT 102(e) date
EXPIRATION DATE:	5 Jun 2015		

	NUMBER	DATE
PRIORITY APPLN. INFO.:	JP 1994-130236	19940613
	JP 1994-173912	19940726
FAMILY INFORMATION:	US 5756339	19980526
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	CHEMICAL	
	GRANTED	

MICROFILM REEL NO: 008452 FRAME NO: 0420
 NUMBER OF CLAIMS: 7

GRAPHICS INFORMATION: 9 Drawing Sheet(s), 18 Figure(s).

AB There is disclosed a **hyperthermostable protease** gene originating in *Pyrococcus furiosus*, in particular, a **hyperthermostable protease** gene encoding the amino acid sequence represented by the SEQ ID NO 1 in the Sequence Listing or a part thereof which retains the activity of the **hyperthermostable protease**. There is also disclosed a process for producing the protease by culturing a transformant transformed with a plasmid into which the above gene has been inserted.

L4 ANSWER 5 OF 11 USPATFULL on STN

ACCESSION NUMBER: 2006:315216 USPATFULL

TITLE: Promoters

INVENTOR(S): Shimojo, Tomoko, Kyoto, JAPAN
 Takakura, Hikaru, Shiga, JAPAN
 Ochiai, Kazuyori, Shiga, JAPAN
 Asada, Kiyozo, Shiga, JAPAN
 Kato, Ikunoshin, Kyoto, JAPAN

PATENT ASSIGNEE(S): TAKARA BIO INC., Shiga, JAPAN (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2006269999	A1	20061130
APPLICATION INFO.:	US 2006-500480	A1	20060808 (11)
RELATED APPLN. INFO.:	Division of Ser. No. US 2003-471868, filed on 15 Sep 2003, PENDING A 371 of International Ser. No. WO 2002-JP2341, filed on 13 Mar 2002		

	NUMBER	DATE
PRIORITY INFORMATION:	JP 2001-72802	20010314
DOCUMENT TYPE:	Utility	

FILE SEGMENT: APPLICATION
 LEGAL REPRESENTATIVE: BROWDY AND NEIMARK, P.L.L.C., 624 NINTH STREET, NW,
 SUITE 300, WASHINGTON, DC, 20001-5303, US
 NUMBER OF CLAIMS: 7
 EXEMPLARY CLAIM: 1
 LINE COUNT: 1278
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.
 AB **Isolated** DNAs which are DNAs having a base sequence
 represented by any of SEQ ID NOS:1 to 6 in Sequence Listing or fragments
 thereof and showing a stationary phase-specific promoter activity in
 gram positive bacteria.

L4 ANSWER 6 OF 11 USPATFULL on STN
 ACCESSION NUMBER: 2004:273688 USPATFULL
 TITLE: Promoters
 INVENTOR(S): Shimojo, Tomoko, Kyoto, JAPAN
 Takakura, Hiraku, Otsu-shi, JAPAN
 Ochiai, Kazuyori, Otsu-shi, JAPAN
 Asada, Kiyozo, Koka-gun, JAPAN
 Kato, Ikunoshin, Uji-shi, JAPAN

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004214185	A1	20041028
	US 7125690	B2	20061024
APPLICATION INFO.:	US 2003-471868	A1	20030915 (10)
	WO 2002-JP2341		20020313
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	BROWDY AND NEIMARK, P.L.L.C., 624 NINTH STREET, NW, SUITE 300, WASHINGTON, DC, 20001-5303		
NUMBER OF CLAIMS:	11		
EXEMPLARY CLAIM:	1		
LINE COUNT:	1294		
CAS INDEXING IS AVAILABLE FOR THIS PATENT.			
AB	Isolated DNAs which are DNAs having a base sequence represented by any of SEQ ID NOS:1 to 6 in Sequence Listing or fragments thereof and showing a stationary phase-specific promoter activity in gram positive bacteria.		

L4 ANSWER 7 OF 11 USPATFULL on STN
 ACCESSION NUMBER: 2002:57586 USPATFULL
 TITLE: Thermostable protease
 INVENTOR(S): Takakura, Hikaru, Otsu, JAPAN
 Morishita, Mio, Otsu, JAPAN
 Shimojo, Tomoko, Kyoto, JAPAN
 Asada, Kiyozo, Shiga, JAPAN
 Kato, Ikunoshin, Uji, JAPAN
 PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Kyoto, JAPAN (non-U.S.
 corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6358726	B1	20020319
	WO 9856926		19981217
APPLICATION INFO.:	US 1999-445472		19991208 (9)
	WO 1998-JP2465		19980604
			19991208 PCT 371 date

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1997-151969	19970610
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	

PRIMARY EXAMINER: Nashed, Nashaat T.
ASSISTANT EXAMINER: Fronda, Christopher L.
LEGAL REPRESENTATIVE: Browdy and Neimark
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 1941

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A **hyperthermostable protease** having the amino acid sequence represented by the SEQ ID NO:1 of the Sequence Listing or a sequence derived therefrom by deletion, substitution, insertion or addition of one to several amino acid residues, a gene encoding the **hyperthermostable protease**, and a process for preparing the protease, aiming at providing by genetic engineering techniques a hyperthermophile protease which is advantageous for industrial use.

L4 ANSWER 8 OF 11 USPATFULL on STN

ACCESSION NUMBER: 2001:112090 USPATFULL
TITLE: Ultrathermostable protease genes
INVENTOR(S): Takakura, Hikaru, Otsu, Japan
Morishita, Mio, Otsu, Japan
Yamamoto, Katsuhiko, Otsu, Japan
Mitta, Masanori, Kyotanabe, Japan
Asada, Kiyozo, Shiga, Japan
Tsunasawa, Susumu, Otsu, Japan
Kato, Ikunoshin, Uji, Japan
PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Kyoto, Japan (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6261822	B1	20010717
	WO 9721823		19970619
APPLICATION INFO.:	US 1998-894818		19980520 (8)
	WO 1996-JP3253		19961107
			19970829 PCT 371 date
			19970829 PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1995-323285	19951212
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Prouty, Rebecca E.	
ASSISTANT EXAMINER:	Rao, Manjunath N.	
LEGAL REPRESENTATIVE:	Browdy & Neimark	
NUMBER OF CLAIMS:	5	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	32 Drawing Figure(s); 24 Drawing Page(s)	
LINE COUNT:	2496	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB There are provided **hyperthermostable proteases** having an amino acid sequences represented by SEQ ID Nos. 1, 3 and 5 of the Sequence Listing or functional equivalents thereof and **hyperthermostable protease** genes encoding those **hyperthermostable protease**. There is also disclosed a process for preparation of a **hyperthermostable protease** by culturing a transformant containing the gene.

L4 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 1999:8129 CAPLUS
DOCUMENT NUMBER: 130:77959
TITLE: Recombinant preparation of mature form of

hyperthermostable proteinase of *Pyrococcus*
furiosus in *Bacillus*
 INVENTOR(S): Takakura, Hikaru; Morishita, Mio; Shimojo, Tomoko;
 Asada, Kiyozo; Kato, Ikunoshin
 PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Japan
 SOURCE: PCT Int. Appl., 82 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9856926	A1	19981217	WO 1998-JP2465	19980604
W: AU, CA, CN, JP, KR, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9875500	A	19981230	AU 1998-75500	19980604
EP 994191	A1	20000419	EP 1998-923114	19980604
EP 994191	B1	20060301		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				
JP 3601835	B2	20041215	JP 1999-502065	19980604
CN 1657614	A	20050824	CN 2004-10101968	19980604
AT 318913	T	20060315	AT 1998-923114	19980604
TW 530088	B	20030501	TW 1998-87109161	19980609
US 6358726	B1	20020319	US 1999-445472	19991208
US 2002132335	A1	20020919	US 2002-90624	20020306
US 6783970	B2	20040831		
US 2005084934	A1	20050421	US 2004-888588	20040712
US 7144719	B2	20061205		
PRIORITY APPLN. INFO.:			JP 1997-151969	A 19970610
			WO 1998-JP2465	W 19980604
			US 1999-445472	A3 19991208
			US 2002-90624	A3 20020306

AB The gene encoding a **hyperthermostable protease** PFUS is isolated from *Pyrococcus furiosus* strain DSM3638 and used for the production of 2 mature forms of protease by expression the gene in *Bacillus*. Mature forms NAPS-1 and SPO-124ΔC comprised of amino acids 133-552 and 133-544 of PFUS, resp., are prepared by transgenic *Bacillus subtilis* strain DB104/pNAPSΔC and strain DB104/pSPO124ΔC. Claimed are methods of recombinant production of the protease by expression of a chimeric gene that also contains the gene encoding the signal peptide of subtilisin.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 10 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:465445 SCISEARCH

THE GENUINE ARTICLE: UR928

TITLE: A **hyperthermostable protease** of the subtilisin family bound to the surface layer of the Archaeon *Staphylothermus marinus*

AUTHOR: Mayr J (Reprint); Lupas A; Kellermann J; Eckerskorn C; Baumeister W; Peters J

CORPORATE SOURCE: MAX PLANCK INST BIOCHEM, D-82152 MARTINSRIED, GERMANY

COUNTRY OF AUTHOR: GERMANY

SOURCE: CURRENT BIOLOGY, (1 JUN 1996) Vol. 6, No. 6, pp. 739-749. ISSN: 0960-9822.

PUBLISHER: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON, ENGLAND W1P 6LB.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 46
ENTRY DATE: Entered STN: 1996
Last Updated on STN: 1996

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: *Staphylothermus marinus*, an archaeon isolated from a geothermally heated marine environment, is a peptide-fermenting, sulphur-dependent organism with an optimum growth temperature of 92 degrees C. It forms grapes of cells, which adhere to each other and to sulphur granules via their surface layer. This glycoprotein layer forms a canopy which is held at a distance of about 70 nm from the cell membrane by membrane-anchored stalks, thereby enclosing a 'quasi-periplasmic space'. Two copies of a globular protease, which probably serves an exodigestive function related to the organism's energy metabolism, are attached near the middle of each stalk.

Results: We have purified and characterized this protease with regard to its enzymatic properties acid thermostability, and have sequenced its gene using an approach based entirely on the polymerase chain reaction. The precursor form is 1345 amino acids long; between residues 64-741, it contains a domain with clear homology to subtilisins, which is interrupted by two large insertions. The enzyme has a broad substrate specificity and a pH optimum of 9.0. It is fully stable from pH 3.2 to 12.7 and is resistant to heat-inactivation to 95 degrees C in the free form acid to 125 degrees C in the stalk-bound form.

Conclusions: This protease is one of the most stable proteases known. Its high resistance towards denaturing agents makes it an interesting target for practical applications. Despite its large size, it is clearly a member of the subtilisin family and represents the only known enzyme that is a stoichiometric S-layer component.

L4 ANSWER 11 OF 11 WPIDS COPYRIGHT 2007 THE THOMSON CORP on STN
ACCESSION NUMBER: 1996-049674 [05] WPIDS
DOC. NO. CPI: C1996-016280 [05]
TITLE: *Pyrococcus furiosus* hyper:thermostable protease gene - useful for recombinant production of hyper:thermostable protease
DERWENT CLASS: B04; D16
INVENTOR: ASADA K; KATO I; MITA M; MITTA M; MORISHITA M; TSUNASAWA S; YAMAMOTO K
PATENT ASSIGNEE: (TAKI-C) TAKARA BIO KK; (TAKI-C) TAKARA SHUZO CO LTD
COUNTRY COUNT: 18

PATENT INFO ABBR.:

PATENT NO	KIND	DATE	WEEK	LA	PG	MAIN IPC
WO 9534645	A1	19951221	(199605)*	JA	85	[8]
JP 08501922	X	19970128	(199714)	JA		[0]
EP 776971	A1	19970604	(199727)	EN	67	[18]
US 5756339	A	19980526	(199828)	EN		
EP 776971	A4	19971229	(199840)	EN		
EP 776971	B1	20011205	(200203)	EN		
DE 69524422	E	20020117	(200213)	DE		
CN 1154717	A	19970716	(200376)	ZH		
JP 2004329217	A	20041125	(200477)	JA	25	
CN 1086737	C	20020626	(200523)	ZH		
JP 3708946	B2	20051019	(200569)	JA	25	

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9534645	A1	WO 1995-JP1095	19950605

CN 1154717 A
 CN 1086737 C
 DE 69524422 E
 EP 776971 A1
 EP 776971 A4
 EP 776971 B1
 DE 69524422 E
 JP 08501922 X
 EP 776971 A1
 US 5756339 A
 EP 776971 B1
 DE 69524422 E
 JP 08501922 X
 JP 2004329217 A Div Ex
 JP 3708946 B2 Div Ex
 US 5756339 A
 JP 2004329217 A
 JP 3708946 B2

CN 1995-194487 19950605
 CN 1995-194487 19950605
 DE 1995-69524422 19950605
 EP 1995-920251 19950605
 EP 1995-920251 19950605
 EP 1995-920251 19950605
 EP 1995-920251 19950605
 WO 1995-JP1095 19950605
 WO 1995-JP1095 19950605
 WO 1995-JP1095 19950605
 WO 1995-JP1095 19950605
 WO 1995-JP1095 19950605
 JP 1996-501922 19950605
 JP 1996-501922 19950605
 JP 1996-501922 19950605
 US 1996-750532 19961213
 JP 2004-188195 20040625
 JP 2004-188195 20040625

FILING DETAILS:

PATENT NO	KIND	PATENT NO
DE 69524422 E	Based on	EP 776971 A
JP 3708946 B2	Previous Publ	JP 2004329217 A
JP 08501922 X	Based on	WO 9534645 A
EP 776971 A1	Based on	WO 9534645 A
US 5756339 A	Based on	WO 9534645 A
EP 776971 B1	Based on	WO 9534645 A
DE 69524422 E	Based on	WO 9534645 A

PRIORITY APPLN. INFO: JP 1994-173912 19940726
 JP 1994-130236 19940613

AB WO 1995034645 A1 UPAB: 20060111

Hyperthermostable protease gene derived from isolated *Pyrococcus furiosus* is new. More specifically, the gene is defined as: (a) encoding the 903 amino acid sequence given in the specification as SEQ.ID.Number 1, or part of the sequence with thermostable protease enzyme activity; (b) having the 2835 bp sequence given in the specification as SEQ.ID.Number 2; or (c) at least part of its sequence (especially the 898 bp sequence given in the specification as SEQ. ID. Number 7) is able to hybridise to one of the sequences (I)-(IV): GGWWSDRRTG TTRRHGTHGC DGTDMTYGAC ACSGG (I) KSTCACGGAA CTCACGTDGC BGGMACDGTG GC (II) ASCMGCAACH GTKCCVGCHA CGTGAGTTCC GTG (III) CHCCGSYVAC RTGBGGAGWD GCCATBGAVG TDCC (IV)

USE - The gene allows recombinant production of a **hyperthermostable protease** (claimed).